Amendments to the Specification

In the following replacement paragraphs, references to *B. odysseyi* 34-hs1^{T} and NRRL B-30641^{T} have been changed to remove the superscript T, changing the relevant portions to *B. odysseyi* 34-hs1 and NRRL B-30641.

Please replace paragraphs 21 through 23 with the following replacement paragraphs:

FIG. 7 is a table illustrating characteristics for differentiating *B. odysseyi* 34-hs1 from related species;

FIG. 8 is a chart illustrating a phylogenetic tree of round-spore forming *Bacillus* and other species closely related to strain 34-hs1 based on maximum likelihood and parsimony analysis of 16S rDNA nucleotide sequences; and

FIG. 9 is a table illustrating DNA-DNA hybridization between *B. odysseyi* sp. Nov. 34-hs1 and related species.

Please replace the paragraph 30 with the following replacement paragraph:

The strain disclosed in this description has been deposited in the Agricultural Research Service Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Ill. 61604, U.S.A., as NRRL B-30641. The deposit was received by NRRL on February 4, 2003, and was given an accession number by the International Depository Authority of NRRL B-30641. The deposit has been made to and received by the International Depository Authority under the provisions of the Budapest Treaty, and all restrictions upon public access to the deposit will be irrevocably removed upon the grant of a patent on this application.

Please replace the paragraph 62 with the following replacement paragraph:

The microbial population of the large surface area of the spacecraft showed, on average (25 determinations), total heterotrophs and spore-formers at 28.0±8.6 and 2.0±1.5 c.f.u. per 25 cm^2, respectively. Isolates were identified by 16S rDNA sequence analysis as species of

Acinetobacter, *Bacillus*, *Curtobacterium*, *Delftia*, *Microbacterium* and *Ralstonia*. Additionally, all fungal isolates were identified as *Aureobasidium pullulans* by 18S rDNA sequence analysis. When purified strains arising from isolated colonies were screened for resistance to harsh conditions, such as UV, gamma radiation, H_2O_2 and desiccation, several spore-forming isolates showed resistance. Of the 45 strains identified, one strain, designated 34hs-1, exhibited distinct spore morphology and was characterized further for its phylogenetic affiliation.

Please replace paragraph 64 with the following replacement paragraph:

Strain 34hs-1 is a Gram-positive, aerobic, rod-shaped, spore-forming bacterium. Cells are 4–5 mm long, 1 mm in diameter and motile. On TSA medium incubated at 32 °C, young colonies are beige, round, ~3 mm in diameter, fairly smooth and flat with entire edges. As shown in FIG. 1, endospores of strain 34hs-1 (1 mm in diameter) are terminal, with one spore per cell, and swell the mother cell.

Please replace paragraphs 66 through 75 with the following replacement paragraphs:

As shown in FIG. 3, ultrathin sections of spores of strain 34hs-1 showed the presence of an exosporium (labeled in FIG. 3 as EX), a spore coat (labeled in FIG. 3 as SC), a cortex (labeled in FIG. 3 as Cortex) and a core (labeled in FIG. 3 as Core).

As shown in FIG. 4, microscopic analyses revealed the partial destruction of 34hs-1 spores by gamma radiation, although remnants of exosporia were left behind; some spores oxidized by H_2O_2 formed 'doughnut-like' structures (Shown in FIG. 5). Further analysis showed highly electron-dense structures in the exosporia (EX) of gamma-irradiated and H_2O_2-treated (Shown in FIG. 6) spores when compared with the untreated control shown in FIG. 3.

(iii) Resistance of spores of strain 34hs-1 to various physical and chemical conditions.

The resistance of *Bacillus* spores to a variety of conditions is well documented. Spores of 34hs-1 exhibited resistance to UV_{254} (254 nm UV radiation), gamma radiation, 5% liquid H_2O_2 and desiccation conditions. Spores of 34hs-1 did not exhibit classic UV_{254} inactivation kinetics: the characteristic 'shoulder' was missing and inactivation did not take effect until well after 400 J

m^{-2}. Spores of strain 34hs-1 exhibited an LD_{90} (90% lethal dose) of ~660 J m^{-2}. Spores of 34hs-1 also survived 0.5 Mrad gamma radiation (0.4% survival). Purified spores exposed to 5% liquid H_2O_2 showed resistance, with nearly 26% of the initial inoculum (1.1×10^7 ml^{-1}) viable after 60 min exposure. Finally, desiccation had no effect on viability of the 34hs-1 spores. When compared with *Bacillus subtilis* strain 168, spores of strain 34hs-1 appeared to be quite resistant, respectively exhibiting 3, 10, 6, and 10 times greater survival when exposed to UV, gamma radiation, H_2O_2, and desiccation, respectively. It is apparent that micro-organisms, now shown to withstand appreciable doses of several sterilants, are present on the surface of a spacecraft being sent to Mars, a pristine extraterrestrial system. Perhaps even more intriguing than their ability to withstand decontamination strategies imposed by mankind is the possibility of such resistances allowing them to survive the highly oxidative UV and gamma radiation-rich environments that they will encounter en route to and on the surface of Mars. This presents a large problem to those concerned with planetary protection, i.e. ensuring appropriate levels of spacecraft cleanliness in order to avoid (i) compromising the integrity of in situ and/or sample-return missions and (ii) contamination of pristine extraterrestrial environments with Earth-derived biomatter.

(iv) Phenotypic characterization.

Strain 34hs-1 grew between 25 and 42 °C, with optimum growth at 30–35 °C, and over the pH range 6–10 (optimum 6–7). It did not require Na^+ for growth. Biochemical characterization of strain 34hs-1 is presented in FIG. 7; where Strain 1 is *B. odysseyi* 34hs-1; Strain 2 is *B. fusiformis* NRRL NRS-350T; Strain 3 is *B. sphaericus* DSM 28T; Strain 4 is *B. pycnus* NRRL NRS-1691T; Strain 5 is *B. neidei* NRRL BD-87T; and Strain 6 is *B. badius* ATCC 14574T. The row in the table labeled "16S rDNA sequence similarity (%)," refers to the percent similarity of the 16S rDNA sequences of each of the shown strains with that of *B. odysseyi* 34hs-1.

This strain produced catalase, but not cytochrome oxidase, gelatinase, urease, tryptophan deaminase, lysine, ornithine decarboxylase, or arginine dihydrolase. It did not show denitrification or acetoin production. 34hs-1 did not ferment glucose or utilize glucose as a sole carbon source. After prolonged incubation (>3 days), arabinose was assimilated; however, this is

not a discriminatory phenotypic trait. Hydrogen sulfide was not produced from thiosulfite. The carbon substrate utilization profile of 34hs-1, as measured by the Biolog system, showed an identification match for *Bacillus badius*. Furthermore, most of the Biolog-generated phenotypic characteristics were similar to those of both *B. sphaericus* and *B. fusiformis* shown in FIG. 7. Strain 34hs-1 did not metabolize common hexoses, pentoses or disaccharides, but preferred pyruvate, amino acids, purine or pyrimidine bases and related compounds as carbon and energy sources. Most round spored *Bacillus* species, including strain 34hs-1, are not able to grow in the absence of oxygen.

(v) Phylogenetic characterization

The 16S rDNA sequences of all known *Firmicutes* were compared with that of 34hs-1. All phylogenetic analyses, based on 16S rDNA sequences, unambiguously demonstrated that 34hs-1 belonged to the low-G+C-containing Gram-positive bacteria. The 16S rDNA sequences of all known members of the Gram-positive bacteria were compared with that of 34hs-1. Bootstrapping (500 replicates) analysis was performed to avoid sampling artifacts. The resulting analyses indicated that 34hs-1 shares a close phylogenetic relationship with *Bacillus* species belonging to rRNA group 2. Neighbor-joining, parsimony and maximum-likelihood analyses were undertaken on this subset of bacteria, using several subdomains of the 16S rDNA. In all analyses, strain 34hs-1 was most closely related to members of the genus *Bacillus*.

Similarities in 16S rDNA sequence between 34hs-1 and closely related *Bacillus* species, recognized by GenBank BLAST searches, were 95–96%. GenBank is a nucleotide sequence database maintained by the National Center for Biotechnology Information, located at 8600 Rockville Pike, Bethesda, MD 20894. Sequence variation of ~3.5% was found between 34hs-1 and *B. fusiformis* ATCC 7055^T and *B. sphaericus* DSM 28^T. A very high sequence variation (8 %) was observed between 34hs-1 and *B. subtilis* ATCC 6633. Such a high degree of dissimilarity within a well-described genus is not uncommon. Likewise, *B. badius*, the strain most phenotypically similar to 34hs-1, was only 91.5% similar in 16S rDNA sequence.

Please replace paragraphs 77 through 79 with the following paragraphs:

The branching order of the phylogenetic tree shown in FIG. 8, showed three distinct clusters, in which one clade contained *Kurthia* species, another group was formed from species of *Sporosarcina*, *Filibacter* and *Planococcus* and a final grouping was composed of species of *Bacillus* and *Caryophanon*, including strain 34hs-1. The round-spore-forming *Bacillus* group was very tightly bound phylogenetically; all members of this clade shared sequence similarities of >95 %. Strain 34hs-1 exhibited the characteristics necessary to place it in *Bacillus* rRNA group 2. To differentiate these closely related species more accurately, DNA–DNA hybridization was performed.

(vi) DNA–DNA hybridization.

As shown in FIG. 9, DNA–DNA hybridization was performed between 34hs-1 and round-spore-forming *Bacillus* and *Sporosarcina* species. The values shown in FIG. 9 are means of at least two determinations between the two selected species. None of the *Bacillus* species that showed very high 16S rDNA sequence similarities (~96 %) exhibited >70% DNA–DNA re-association values with 34hs-1, i.e. the cutoff value required to place strains within the same species. In particular, the hybridization value between 34hs-1 and *B. silvestris* NRRL B-23336T was only 17 %, whereas their 16S rDNA sequences were 96.4% similar. Also, strain 34hs-1 and *B. sphaericus* NRRL BD-113 showed 17% DNA–DNA hybridization, but ~97% 16S rDNA sequence similarity. Based on DNA–DNA re-association values, strain 34hs-1 represents a novel Bacillus species, *Bacillus odysseyi* sp. nov.

Please replace paragraph 82 with the following paragraph:

Cells are rod-shaped, 4–5 mm in length, approximately 1 mm in diameter and motile. Furthermore, the *B. odysseyi* cells are Gram-positive and aerobic, form terminal endospores, and the spores show an additional exosporium layer. Colonies on TSA are round, smooth, flat with entire edges and beige in color. Sodium ions are not essential for growth; growth occurs in 0–5% NaCl. *B. odysseyi* grows at pH 6–10 (optimum at pH 7) and 25–42 °C (optimum 30–35 °C). With the exception of arabinose, breakdown of sugars to acids does not occur following prolonged incubation. *B. odysseyi* prefers pyruvate, amino acids, purine or pyrimidine bases and related compounds as carbon and energy sources. *B. odysseyi* is catalase-positive, but does not produce gelatinase, arginine dihydrolase, lysine or ornithine decarboxylase, lipase, amylase or

alginase. The organism does not produce H_2S from thiosulfite and is not involved in denitrification. The type strain, strain 34hs-1 (=ATCC PTA-4993^T= NRRL B-30641=NBRC 100172^T), was isolated from the surface of the Mars Odyssey spacecraft.